United States Department of Agriculture
Agricultural Marketing Service
Science & Technology
Monitoring Programs Office
8609 Sudley Rd., Ste. 206
Manassas, VA 20110

May 16, 2005

TO: See Distribution List

FROM: Martha Lamont, Director
Monitoring Programs Office

SUBJECT: Microbiological Data Program Plan, July through December 2005

This Program Plan serves as the current Statement of Work for the period July 1, 2005, through December 31, 2005, for each State participating in the Microbiological Data Program (MDP). This document also stipulates work assignments for the Federal facility participating in MDP.

I. ADMINISTRATIVE UPDATES

A. Personnel: Program participants are reminded to keep MDP management informed of any critical equipment purchases, staffing issues, or expected increases in rent or sample turn-around-time (e.g., due to laboratory or office renovation/relocation). This information is required under the terms of MDP Cooperative Agreements (Section II, Responsibilities) between USDA and participating States.

B. Summary Status: The 2004 MDP Data Summary is being drafted by Monitoring Programs Office (MPO) staff.

C. MPO Personnel: Assignments have been rotated according to standard procedures and were effective May 1, 2005. Terry Councell has been tasked with managing the Pesticide Data Program Water Project.

Laboratory Liaisons

Jan Doyle	Michigan, Ohio, Wisconsin
Donna Dickriede	California, Colorado, Florida
Shanker Reddy	Minnesota, New York, Washington
John Punzi	National Science Laboratory

D. Financial/Cooperative Agreements: The signature process for the MDP Cooperative Agreements for Fiscal Year (FY) 2005 has been completed. Funds have been obligated and States should begin billing FY05 expenses.

E. MDP Program Meetings: MPO plans to hold a joint Federal/State Meeting for the Pesticide Data Program (PDP) and Microbiological Data Program (MDP). Anticipated

meeting dates and location are September 27-29, 2005, in Denver, Colorado. Administrative, sampling, and technical issues for PDP and MDP will be covered. Required attendees are Administrative, Sampling, and Technical Program Managers for each program. Further information and a draft agenda will be distributed at a later date.

F. Electronic Transfer of Data:

RDE Version Upgrades: A version upgrade for the Web-based Remote Data Entry (RDE) system was installed in February 2005. The Build 7 upgrade included the addition of new data tables and data entry screens for capturing multiplex Polymerase Chain Reaction (mPCR) findings for *E. coli* and a new positive produce control in the QA results. A version upgrade is planned for June 2005 to fix several identified glitches. A version upgrade for the RDE e-SIF system for laptops/palmtops is planned for September 2005. MPO maintains a Change Request Database to capture all problems identified and suggestions made regarding the RDE system.

RDE Web Addresses: RDE users in the laboratories should be using the SSL (Secure Socket Layer) site address to access the Web-based RDE system on the AMS production Web server. The only difference is the addition of the letter "s" following "http". This SSL technology is used to encrypt all data passed between the user's computer and the central web server. If the secure site is not available, the AMS developmental Web server can be used.

II. PROGRAM SAMPLING AND TESTING UPDATES

A. Sampling: Shipping Charts are distributed quarterly to Sampling Managers by MPO.

Cantaloupe, tomatoes, green onions, parsley/cilantro, and lettuce (leaf or romaine) will continue until September 30, 2005, when parsley/cilantro will be discontinued and alfalfa sprouts will be added. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to the AMS National Science Laboratory [NSL (US4)]. All other samples will be analyzed by the laboratory for that collection State.

B. Testing: Cantaloupe, tomatoes, green onions, parsley/cilantro, and lettuce (leaf or romaine) will continue until September 30, 2005 when parsley/cilantro will be discontinued and alfalfa sprouts will be added. Samples collected in Maryland will be tested by the Ohio laboratory (OH4) and those collected in Texas will be tested by NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

Target Microorganisms: MDP laboratories will continue quantitative testing of all samples for *E. coli* using ColiComplete. Method procedures are detailed in Standard Operating Procedure (SOP) MDP-MTH-01, *Escherichia coli* Most Probable Number Method.

Participating laboratories will continue to test Lauryl Sulfate Tryptose broths, which resulted from SOP MDP-MTH-01, for pathogenic *E. coli* by mPCR according to SOP MDP-MTH-07. Results are entered in the RDE system using new mPCR data entry screens that were added with the Build 7 upgrade in February 2005. Results associated with data sets from January through May 2005 that have already been transmitted to

MPO are entered and submitted to Amsmpo.data@usda.gov using an MPO-provided Excel file. Laboratories that import data to RDE shall submit mPCR results to MPO at Amsmpo.data@usda.gov using MPO-provided Excel or Access files until such time as the RDE import function is modified to include the mPCR data. Electronic copies of the gels shall also be sent to Diana Haynes, MPO, at diana.haynes@usda.gov. The data will be qualified by MPO until the individual laboratory has received a letter stating that the staff has attained sufficient expertise in the procedures. At that point, copies of the gels need no longer be sent for data qualification.

MDP laboratories will continue to screen all samples for *Salmonella* (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-04, BAX® System for Detection of *Salmonella* in Fresh Produce. Presumptive positive samples are subjected to enrichment and isolation as described in SOP MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce.

MDP laboratories will continue to screen all samples for *E. coli* O157:H7 (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX® System. Presumptive positive samples are subjected to immunomagnetic separation (IMS) procedures and confirmed culturally, as described in SOP MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Identification.

C. Quality Assurance:

Proficiency Testing Program: A standardized proficiency round for MDP laboratories for *Salmonella* was conducted in December 2004. Lyophilized culture test kits were supplied by the American Type Culture Collection (ATCC) to each MDP laboratory Quality Assurance Officer (QAO) to prepare samples for testing. Each kit contained lyophilized microorganisms of *Salmonella typhimurium,* a negative culture control, and blank controls. All inocula were randomly assigned designation numbers specific to each kit. Samples of cantaloupe, leaf lettuce, and parsley were inoculated by the QAO and then transferred to the MDP Technical Program Manager for analysis. Samples were analyzed according to MDP SOPs and results for the tests were recorded electronically on an interactive reporting form. The tests included BAX®-PCR screening followed by isolation of *Salmonella* from BAX® -positive samples. Isolates were confirmed by serotyping. All MDP laboratories demonstrated acceptable performance in the *Salmonella* proficiency test.

The next proficiency test, which will include a surrogate *E. coli* strain that can be used in place of *E. coli* O157:H7, is currently under development. The actual date of this proficiency test will be announced for the fourth quarter. MPO and participating laboratories are pursuing registration with the Biodefense and Emerging Infections Research Resources Repository (BEI Resources) in order to obtain *E. coli* O157:H7. This strain is no longer available through ATCC but may be required for future control strains and proficiency testing.

mPCR Method Tryout: In March and April 2005, laboratories participated in a two-pronged method tryout to detect Shiga toxin-producing *E. coli (*STEC) and

Enterotoxigenic *E. coli* (ETEC) in spiked and unspiked produce samples using an mPCR protocol. This protocol was developed specifically for MDP by Food and Drug Administration (FDA)/Center for Food Safety and Nutrition (CFSAN) and validated by the Florida laboratory. All of the participating laboratories were able to extract, amplify, and separate target DNA bands for STEC and EHEC. However, MPO is reviewing the gels to provide feedback to the laboratories. The feedback will be individualized for each laboratory's specific electrophoretic systems and will enhance the quality of their results. This tryout resulted in the development of SOP MDP-MTH-07.

SOPs: SOPs are posted to the MDP website when distributed to program participants. http://www.ams.usda.gov/science/MPO/SOPs.htm.

The following SOPs were distributed February 1, 2005

- MDP-LABOP-08, Procedure for Testing and Maintaining Control Strains (Revision 02)
- MDP-MTH-01, *Escherichia coli* MPN Method (Revision 04)
- MDP-MTH-03A, Isolation and Identification of *Salmonella* from Fresh Produce (Original)
- MDP-MTH-04, BAX® System for Detection of *Salmonella* in Fresh Produce (Revision 01)
- MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX® System (Revision 01)
- MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Identification (Revision 01)
- MDP-QA-03, Quality Assurance (QA) Controls (Revision 02),
  Attachment 1, Current QA Control Strain Information
  Attachment 2, QC Control Failure Reporting Form

The following SOP and attachment were archived February 1, 2005

- MDP-MTH-03, *Salmonella* Cultural Method (Revision 02)
- MDP-QA-03 Attachment 01, tabbed worksheet labeled "Historical" was archived. Attachment 01 now consists of a single spreadsheet entitled "Current" that presents the QA strains currently used in SOPs.

The following SOPs were distributed May 1, 2005

- MDP-MTH-07, mPCR Screening for Pathogenic *E. coli* (Original)
  Attachment 01, mPCR Validation Protocol
- MDP-MTH-02, *Salmonella* VIDAS® Method (Revision 03)

D. Archiving and Additional Testing:

Archival of Isolates: NSL, Gastonia, NC has been established as a centralized location for archival of isolates as well as a distribution center for isolates from MDP testing laboratories to the reference laboratories.

Additional Testing by Reference Laboratories: Pathogenic *E. coli* isolates [i.e. those determined to contain genes coding for Shiga toxins (Stx-1 and Stx-2) in STEC and the heat labile (LT-1) and heat stable (ST-1) toxins in ETEC] are shipped by NSL to Pennsylvania State University for serotyping and to the FDA/Center for Veterinary Medicine (CVM) laboratory in Laurel, MD for antimicrobial resistance testing and inclusion in the National Antimicrobial Resistance Monitoring System (NARMS) and pulsed-field gel electrophoresis (PFGE) analysis for inclusion in PulseNet. *Salmonella* and *E. coli* O157 isolates are frozen in two Microbank™ vials which are shipped to NSL. Isolates are then shipped to FDA/CVM for antimicrobial resistance testing and inclusion in NARMS, PFGE for inclusion in PulseNet, and serotyping.

AMS will transfer data to Centers for Disease Control and Prevention (CDC) and FDA on a semi-annual basis.

E. Future Program Directions:

Universal Pre-enrichment Broth (UPB): Current MDP methods use different pre-enrichment broths for growing target bacteria (lactose broth for *Salmonella* and mEC + novobiocin for *E. coli* O157:H7). Inclusion of new target organisms, such as *Shigella*, may require additional enrichment broths. Using a single pre-enrichment broth such as UPB for culturing different bacteria will streamline this labor-intensive step and subsequent analyses in the screening process. Recent studies have demonstrated that UPB provides improved enrichment of stressed and sub-lethally injured cells of some pathogenic bacteria of interest to MDP. MPO is collaborating with the Division of Consolidated Laboratory Services (DCLS), Richmond, VA on UPB method development work. DCLS is also testing the feasibility of using UPB as a wash eluent instead of BPW+ 0.1% Tween and investigating the use of a DNA purification step prior to BAX analysis. Pending method validation, MPO anticipates implementation of UPB in January, 2006.

Sprouts (alfalfa/clover and mung bean) are being investigated as a replacement commodity for cilantro/parsley in October 2005. MPO is collaborating with DCLS to investigate the optimum stomaching conditions and the effect of stomaching on the 4-methylumbelliferyl β-D-glucuronide (MUG) assay for *E. coli* (SOP MDP-MTH-01). Sprouts will be tested for all target organisms.

Shigella: Four *Shigella* species, A-D, (*dysenteriae, flexneri, boydii* and *sonnei*, respectively) have been identified as human pathogens and are therefore of interest to the MDP program for screening purposes. MPO is collaborating with DCLS in developing a PCR-based detection method for *Shigella* which will include enrichment and DNA clean-up, and will be followed by cultural isolation and identification steps. Initial method performance studies were investigated by the Minnesota laboratory. Prior to program-wide introduction, all methods will be verified by the participating laboratories. Pending method development, MPO anticipates addition of *Shigella* as a target organism in calendar year 2006.